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Laguncularia racemosa top-layer sediment microorganism makeup in relation to differing levels of perceived anthropogenic impact in Bocas del Drago, Bocas del Toro, Panama

Gabrielle Glendening
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***Laguncularia racemosa* top-layer sediment microorganism makeup in relation to differing levels of perceived anthropogenic impact in Bocas del Drago, Bocas del Toro, Panama**

Independent Study Project
SIT Panama: Tropical Ecology, Marine Ecosystems, and Biodiversity Conservation
Fall 2022

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I. Abstract

As mangrove forests are destroyed by human factors across the earth, many crucial ecological processes that take place in these systems of trees are obstructed. One of the most important roles played by mangroves is their ability to sequester carbon in the sediment, as this storage of carbon helps diminish atmospheric warming. Many sediment microorganisms help in this process of carbon sequestration and play various other vital roles in mangrove ecosystems. Microorganisms in marine sediments can be used to assess the health of the surrounding environment. Past research has found significant differences in sediment microorganism composition, abundance, and diversity in relation to varying levels of human pollution. This research aimed to observe the differences in microorganism makeup in *Laguncularia racemosa* sediment in Bocas del Toro, Panama, between sites of perceived low, medium, and high anthropogenic influence. The data collected was not statistically significant due to the limited scope and lack of controlled environment, but revealed a difference in bacterial abundance, microorganism percent composition, diversity, and evenness between the three sites of perceived low, medium, and high anthropogenic impact. There was also a difference in average biomass of bacteria and protozoa observed between the three sites. These results differ from past literature on the topic of specific increases or decreases of these factors, but align with research asserting an observed difference in marine sediment microorganism composition and abundance between sites of varying levels of anthropogenic impact. Future studies may quantify and isolate relationships between sediment microorganisms and environmental pollution levels.

II. Acknowledgements

I would like to thank my research advisor, Gabriel Jácome, for his invaluable advice, support, and enthusiasm for this project. I additionally want to express my gratitude to Aly Dagang for her guidance, kindness, and teachings throughout this semester. You both opened my eyes to new perspectives, pushed me to realize my scientific potential, and unwaveringly encouraged my passions. I also extend my thanks to the staff at Yarisnori for housing, feeding, and caring for me while I collected my samples. Lastly, I want to thank my fellow SIT students for being a source of support and friendship throughout my time in Panama. I especially extend my gratitude to Skye McDonald for being my Bocas buddy and lending me unconditional encouragement, company, and comradery during our ISPs.

Table of Contents

I. Abstract	1
II. Acknowledgements.....	2
III. Introduction.....	4
<i>i. Mangrove Ecology</i>	
<i>ii. Mangroves Globally</i>	
<i>iii. Blue Carbon</i>	
<i>iv. Mangrove Carbon Sink</i>	
<i>v. Mangrove Sediment Microbial Makeup and Microorganism Roles</i>	
<i>vi. Mangrove Sediment Diversity in Terms of Environmental Factors</i>	
<i>vii. Mangrove Sediment Diversity in Terms of Human Impact</i>	
<i>viii. Sediment Microorganism Composition and Environmental Health</i>	
<i>ix. Mangroves Locally</i>	
<i>x. Site of study</i>	
IV. Research Question.....	7
V. Methods	8
VI. Ethics	9
VII. Results	9
<i>i. Percent Composition</i>	
<i>ii. Diversity and Evenness</i>	
<i>iii. Statistical significance</i>	
<i>iv. Abundance & Biomass</i>	
VIII. Discussion	15
<i>i. Percent Composition</i>	
<i>ii. Diversity and Evenness</i>	
<i>iii. Abundance & Biomass</i>	
IX. Conclusion	17
X. Works Cited	19
XI. Appendix	25
<i>i. Photos</i>	
<i>ii. Volume of Sample in Field of View Calculations</i>	

III. Introduction

Mangrove Ecology

Mangrove ecosystems are defined by highly adapted trees growing in intertidal environments (Feller et al. 2010). Of their features, the tree's salinity tolerance is perhaps most notable. Depending on the species, mangroves can tolerate salinities ranging from fresh water to hypersaline environments exceeding 100 parts per thousand (Romañach et al. 2018). This special adaptation allows the trees to live in coastal environments where many plants would not survive, creating a unique ecosystem connecting the ocean and the land that hosts a huge variety of organisms. Furthermore, mangrove forests play a crucial role in protecting coastlines from storms and naturally occurring erosion (Lovelock et al. 2005).

Mangroves Globally

Mangrove forests are found throughout the Americas, Asia, Africa, and Oceania (Alongi 2002). This distribution spans 118 countries (Schmid & Tomlinson 1987). Globally, mangroves are found along 25% of all coastlines, and along 75% of tropical coastlines. According to a 2018 study, this makes up 8,349,500 ha of earth's surface (Romañach et al). There are around 54 species of mangroves globally (Alongi 2002).

In some parts of the world, more than 50% of mangrove habitats have been lost (Romañach et al. 2018). A 2001 study asserted that just between the 1980s and 1990s, more than 35% of the earth's mangroves were destroyed (Valiela et al. 2001). The losses of these important coastal ecosystems can be primarily attributed to anthropogenic impacts. It has been estimated that between 2000 and 2016, up to 62% of mangrove forest destruction worldwide was due to land use change, often for agricultural purposes (Goldberg et al. 2020). An emphasis on conservation has slowed these losses in recent years, but the historical destruction has monumentally impacted mangrove populations across the globe (Goldberg et al. 2020).

Blue Carbon

While mangrove forests only make up ~2% of marine environments, they account for anywhere between 10 and 15 percent of blue carbon burial (Sasmito et al. 2020). Blue Carbon is a term used to refer to atmospheric carbon captured and stored by marine environments, including both ocean and coastal habitats (Macreadie et al. 2019). Blue carbon storage helps combat rising levels of atmospheric carbon that contribute to warming earth temperature. Of marine ecosystems, mangroves, sea grasses, and salt marshes sequester carbon at the fastest rate. (Sasmito et al. 2020). Over recent years, studies have highlighted the role that these high levels of blue carbon sequestration play in combating global climate change (Jennerjahn 2021).

Mangrove Carbon Sink

Of the biogeochemical processes that occur in these habitats, many consider mangrove's carbon sequestration abilities of extremely high importance (Alongi 2012). As global climate change progresses and levels of atmospheric greenhouse gasses increase, mangroves, and wetlands in general, act as the largest terrestrial biological component in stably storing blue carbon in above and below ground biomass (Allard et al. 2020, Chmura et al. 2003).

While rates and amounts of carbon storage can be highly variable even within a single mangrove system, mangroves can store up to 10 times more carbon per hectare than terrestrial forests (Alongi 2012). This process of carbon sequestration happens as mangrove trees use

carbon from atmospheric carbon dioxide for life sustaining cellular processes. When leaves and branches fall from the trees and are buried in the soil, the carbon stored in these structures is transferred into the mangrove sediment (Sasmito et al. 2020).

In a mangrove biome, an estimated average of 937 tC ha⁻¹ of carbon is stored in sediment and roots (Alongi 2012). A large part of carbon sequestration in mangrove soil is dependent on microbial interactions such as high primary production and low rates of decomposition of soil organic matter (Kim et al. 2021). While sedimentary microbial processes are vital to blue carbon sequestration, a large amount of research has not been conducted on them in comparison to those in many other ecosystems.

Mangrove Sediment Microbial Makeup and Microorganism Roles

Containing as much biomass as seawater across the globe, marine sediment in general covers around 70% of the earth's surface (Hoshino et al. 2020). Recent characterization studies of mangrove sediments have noted high species richness and Shannon diversity in bacteria, followed by lower levels in archaea, and the lowest in fungi (Cheung et al. 2018). In respect to bacteria, mangrove fungal communities have had very few studies published concerning their diversity or activity (Ghizelini et al. 2012).

In general, bacteria comprise most of the biomass in all types of sediments (Nealson 1997). The kingdom's species richness in marine sediments is high, comparable to the richness in topsoil and the richness in seawater (Hoshino et al. 2020). Bacteria play countless roles in sediment, but most notably act as “chemists” and make up the majority of chemical activity across sediments of all varieties (Nealson 1997). Bacterial activity creates electron donor and electron acceptor profiles typically seen in sediment, forming vertical nutrient outlines (Nealson 1997). Bacteria support not just soil health, but act as pillars for the entirety of the mangrove forest and the various ecosystems it connects to. Through numerous chemical processes, bacteria are understood to release nutrients into the water surrounding mangrove systems and therefore establish the base of the local marine food chain (Halder & Nazareth 2018).

Fungi found in sediment play important roles in maintaining ecosystem balance. The most critical functions include nutrient cycling and food web support (Amend et al. 2019). Of the millions of species of fungi found on the planet, only a relatively small portion are found in marine ecosystems such as mangrove sediment (Amend et al. 2019). In terms of species richness, little data has been collected, but it has been found that mangrove fungal communities are differentiated by spatial and host structural factors (Lee et al. 2019). It has been hypothesized that fungi play a role in the chemistry of marine sediments, along with being a part of carbon sequestration. They can also often be associated with macroorganisms in marine environments, acting as commensals or pathogens (Amend et al. 2019).

Protozoa in sediment are often associated with nutrient recycling of detritus and other mineral nutrients (Pratt & Cairns 1985). A study focusing on a mangrove forest in India found evidence of nanoflagellates, microciliates, macrociliates, and nematodes connected to leaf litter degradation processes (Padma et al. 2003). While a large amount of data has not been published on the richness of marine sediment associated protozoa, a 1998 study found between 597 and 793 species in this category (Finlay 1998).

Mangrove Sediment Diversity in Terms of Environmental Factors

There have been distinct differences in mangrove sediment microorganism community makeup observed in respect to many unique environmental factors. Within one community, it has

been revealed that fungal and bacterial diversity can fluctuate along soil-root interfaces (Zhuang et al. 2020). Beyond this observation of a smaller scale, other studies have highlighted the clear cause and effect of cycling environmental factors such as wet and dry seasons, ocean temperatures, or naturally occurring soil moisture content on changes in sediment organism diversity (Cheung et al. 2018, Treves et al. 2012). Abundance, richness, and diversity of benthic protozoa were all found to differ between wet and dry seasons of a specific mangrove forest (Chen et al. 2018). These patterns of spatial- and temporal-defined changes in microbial communities hold consistent for most types of forest sediment, with differences in soil microorganisms observed having correlation to naturally occurring processes and niches.

It has been revealed that in marine sediments, oxygen presence or absence and organic carbon concentration are some of the most important factors influencing microbial composition (Hoshino et al. 2020). In relation to these chemically measured factors, a boreal forest soil study demonstrated bacterial variability relating to pH and soil moisture (Dimitriu et al. 2010). As many bacteria are highly specialized for environments of varying salinity, pH, oxygen content, etc., it follows accepted scientific thought for changes in the biochemical makeup of the soil to result in differing microorganism inhabitants.

Not only does the chemical profile of the sediment influence the microorganism inhabitants, but a correlation has been found between host community structure and forest sediment microbial composition. In the Caribbean region, Troxler et al. (2012) found that bacterial community structure in soil can be clearly associated with the natural canopy community structure in tropical peatland. During mangrove succession, it has been found that protozoan community makeup and abundance change significantly (Chen et al. 2018). In a single community of trees, sediment microbial communities can differ based on naturally occurring chemical, physical and temporal factors.

Mangrove Sediment Diversity in Terms of Human Impact

With threatening levels of pollution and climate change, researchers have begun to examine the relationships between human induced detrimental effects and microorganism composition in mangrove ecosystems. In broad biogeochemical terms, the increasingly anaerobic soil conditions found in some mangrove soils due to pollution can turn usually environmentally beneficial microbial carbon processes into damaging greenhouse gas emitters, although the effect is historically understood to be the opposite (Shiau & Chiu 2020).

Regarding microbial variability, changes in soil composition, water salinity, and other human-induced environmental stressors have been observed to shift the microbial makeup in mangrove peat soil (Chambers et al. 2016). Specific studies on oil contamination in mangrove forests have revealed significant changes in microbial community makeup before and after the contamination event, demonstrating specifically the existence of an effect of nitrate stimulation on microbe population composition (Wang et al. 2016, dos Santos et al. 2011). Pesticide contamination and waste water introduction have also been found to induce multidirectional changes in sediment microbial community composition (Widenfalk et al. 2008, Saarenheimo et al. 2017).

Pollution in general has been found to significantly affect marine sediment microorganism composition. In looking at anthropogenic effects on sediment fungi populations, more polluted sites have been identified to have higher species richness than areas of better natural preservation (Ghizelini et al. 2019). Total bacterial and protozoa counts have been found to be significantly higher in non-polluted sediments (Tso & Taghon 1997). When considering

microplastics, one of the largest sources of pollution in the ocean that often contaminates mangrove forests, there are clear effects on mangrove sediment microbial diversity and richness (Chen et al. 2022).

Sediment Microorganism Composition and Environmental Health

Microorganisms in marine sediment can provide researchers with valuable information about the health of the environment. In general, microbial toxicity tests are a highly regarded tool used as indicators of soil or sediment pollution (van Beelen & Doelman 1997). Studies have used bacterial diversity as an indicator of sediment pollution levels to determine the surrounding environmental health (Ford et al. 2005). Protozoa have additionally been utilized to appraise sediment quality in mangrove ecosystems (Chen et al. 2008). In recent years, microorganisms have become a promising source of surveying and monitoring ecosystem health specifically in wetlands because of the ability of a microbial community to change rapidly and significantly in response to relatively small physical or chemical factors (Sims et al. 2013).

Mangroves Locally

Mangrove forests are one of the most important ecosystems for environmental conservation both worldwide, and more specifically, in the Caribbean (Harborne et al. 2006). In the Caribbean region, mangrove forests inhabit around 9800 km² of the coastal zone (Lovell et al. 2005). In Bocas del Toro specifically, most of the mangrove forest system consists of shrub or dwarf trees with the dominant species being *Rhizophora mangle*, commonly known as red mangroves (Guzman et al. 2005). These mangroves interact with a network of coral reefs and seagrass throughout the coastal area of the region (Guzman et al. 2005). In Bocas del Toro, mangroves are facing similar threats as they do across the globe. A 2008 study determined that mangrove clearing for anthropogenic reasons in the Panamanian Caribbean induced multiple abiotic and biotic factors throughout the associated ecosystems in the region (Granek & Ruttenberg 2008). There have been no studies concerning mangrove sediment microorganism composition in or near the area of study.

Site of Study

Bocas del Toro is a province in northwest Panama bordered by the Caribbean sea on its northern coast. This region of the world is considered neo-tropical, with an estimated 68% of the province covered in rainforest (Guzman et al. 2005) The region of Bocas, especially the islands of Isla Colón and Bastimentos, receives high levels of international tourism throughout the year (Kayes 2005). Bocas del Drago, the site of study for this research, is a strip of coast along the northern side of Isla Colón, about 15 km from the town of Bocas del Toro. The area of study includes three sites along the coast near Bocas del Drago, ranging from a highly visited tourist beach to a rarely visited strip of forest.

IV. Research Question

Does sediment microorganism composition from fringe populations of *Laguncularia racemosa* differ in relation to areas of perceived low, medium, and high levels of anthropogenic impact in Bocas del Drago, Bocas del Toro, Panama?

V. Methods

Sediment samples were collected from 3 sites of fringe *Laguncularia racemosa* populations along a strip of coast in Bocas Del Drago, Bocas Del Toro, Panama. The 3 sites were of varying levels of human interference/pollution. The site designated for high human interference was on Playa Estrella, a popular tourist destination with many restaurants and boats on the shore. The mangrove area was observed to be polluted by human trash such as plastic bottles, styrofoam, and cans, with many human walking paths running through the trees. The site of medium human interference was close to Bocas Del Drago, where there are more occasional boats and the buildings are set further from the mangroves, but there are still people traversing through the area. This area contained trash, but more sparsely distributed, and had a human walking path running next to the forest but not through it. The site of low human interference was between Bocas Del Drago and Playa Estrella, an area of the mangrove fringe where human activity is rare. There was rarely trash observed, and was no path close to the forest.

For each of the three sites, 9 core samples total were collected. Three general areas were identified in each sample site along the fringe- beginning, middle, and end, running from start to finish of the designated area (with about 10 m between those sites). Standing in each general area, a core of 10 cm in diameter was randomly tossed (Protocol: Seagrass Core Biomass 2020). The random toss was repeated three times in each area (beginning, middle, end) of the study segment. When the core landed and the specific sample site was identified, the core sampling began. The core was worked down 5-10 cm into the sediment, and then slowly tilted in the typical manner of core sampling. The bottom of the core was covered by hand as the core was pulled up in order to contain the sediment, and the sediment was collected in a ziplock bag.

Once brought back to the data collection area, the sample was thoroughly homogenized in its ziplock bag by vigorously shaking and spinning it by hand. The sample was then placed in a pre-weighed aluminum tray and the weight of the wet sample was recorded. A 5 g section from each core was then suspended in 145 mL of water. The rest of the sample was then set out to dry in the sun. Once fully dried (after 2 to 3 days), the dry weight of the sample was recorded, and the percentage of water weight lost was calculated.

From each of these samples, approximately 10 microliters (a drop from a pipette) was put on a slide and a wet mount was prepared. (Preparing a wet mount - Centers for Disease Control and Prevention). In order to represent the whole sample, 3 slides were made per core. The slides were then examined at 100x. In each slide, 3 fields of view were randomly selected by moving the slide haphazardly on the stage by hand. For each of the three fields of view, an iPhone was used to take a picture through the microscope eyepiece. This gave 27 pictures in total for each of the 9 sub-sites, or 81 pictures per each of the three main areas.

The pictures were each then analyzed for the number of bacteria, number of fungi, and number of protozoa. Due to their often inconsistent shapes, fungi and protozoa were counted by hand (Figure 6, appendix). Because of the higher quantity, bacterial numbers were counted using ImageJ, a software that can automatically recognize dots of a certain programmed size (Al-Osta et al. 2021).

Once the numbers were collected for each picture, the average number of bacteria, fungi, and protozoa were calculated. The total sum of each group was additionally calculated, and percentages of each group per site were calculated. To calculate biomass, an estimation was taken of volume of sample in field of view. For each core, the average amount of bacteria, fungi, and protozoa in each field of view was calculated. For this study, an estimated 5.78×10^{-10} g of wet sediment sample was visible in each field of view (calculations included in appendix a).

Using this number, it was possible to calculate the amount of bacteria, fungi, and protozoa present per gram of mangrove sediment for each core and each area respectively. The biomass was calculated individually per sample using both the percent weight lost and the wet weight, and the average was then taken for these biomasses respectively. Single factor ANOVA, multiple factor ANOVA, and a linear regression were run to assess statistical significance of the data.

VI. Ethics

Before beginning any data collection, the research proposal for this study was reviewed and approved by the Institutional Review Board (IRB). This study did not involve human participants and did not include any interviews, therefore not invoking those areas of ethical concern in the IRB process. In the case of this project, the ethical concerns dealt with environmental interactions and resource use. Every effort was made to limit harm or disturbance to the ecosystem when collecting samples. This included avoiding damage to any plants or roots by treading carefully in the mangrove forests, leaving no trace of trash or pollution behind, and not using any chemicals on my body or on my materials that could end up in the environment. I additionally only took small samples of sediment in an effort to leave the ecosystem mainly untouched. Beyond these ethical sampling efforts, my stay in Bocas del Toro put stress on a community already struggling for resources. In order to minimize my impact, I practiced environmentally conscious living standards of using as little fresh water as possible through taking brief showers, bringing only biodegradable soaps, not using air conditioners, and minimizing electricity use.

VII. Results

Percent Composition

The low anthropogenic impact site sediment contained 99.87% bacteria, the medium site contained 99.89% bacteria, and the high site contained 99.91% bacteria. This correlated to a trendline of $R^2=1$. The low anthropogenic impact site contained 0.00098% fungi, the medium site contained 0.00081% fungi, and the high site contained 0.00072% fungi. This correlated to a trendline of $R^2=0.969$. The low anthropogenic impact site contained 0.00028% protozoa, the medium site contained 0.00024% protozoa, and the high site contained 0.00016% protozoa. This correlated to a trendline of $R^2=0.964$ (Figure 1).

Site	Percent Bacteria	Percent Fungi	Percent Protozoa
Low Anthropogenic Impact	99.87%	0.00098%	0.00028%
Medium Anthropogenic Impact	99.89%	0.00081%	0.00024%

High Anthropogenic Impact	99.91%	0.00072%	0.00016%
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Table 1. Percent microorganism composition of three sites of differing anthropogenic impact.

Microorganism Composition Across 3 Sites

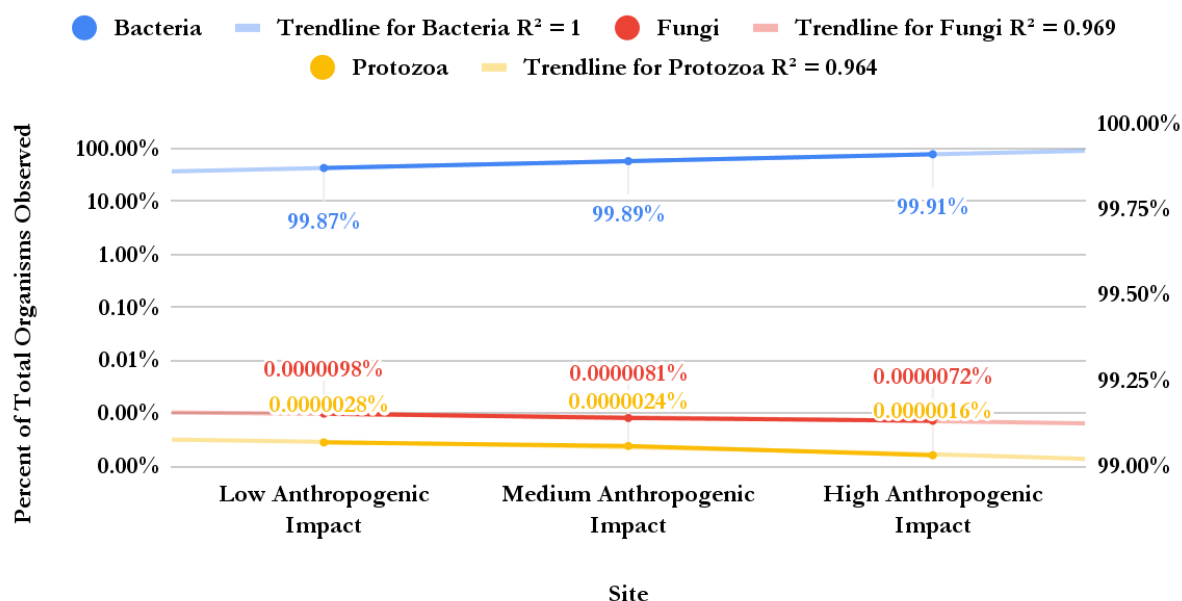


Figure 1. Microorganism composition by percentage at sites of low, medium, and high anthropogenic impact. Bacteria associated with right vertical axis, fungi and protozoa associated with left vertical axis.

Diversity and Evenness

Diversity and evenness in terms of the three groups of bacteria, fungi, and protozoa were found using Shannon Diversity index and Shannon evenness calculation. The site of low anthropogenic impact was found to have the highest diversity and evenness, with the site of medium anthropogenic impact having the next highest of both of these values, and the site of high anthropogenic impact having the lowest diversity and evenness. The typical range for Shannon diversity is 1.5-3.5. Shannon evenness ranges from 0 to 1 where 1 equals 100% evenness of groups.

Site	Shannon Diversity Index	Shannon Evenness
Low Anthropogenic Impact	0.01033016344	0.009402919979
Medium Anthropogenic Impact	0.008399065776	0.007645159136
High Anthropogenic Impact	0.007501358716	0.006828030956

Table 2. Shannon diversity and Shannon evenness indices for sites of low, medium, and high anthropogenic impact.

Statistical significance

For bacterial numbers, a single factor ANOVA test was performed, and there was found to be a statistically significant difference between the three sites of differing impact ($p = 0.002$). A multiple factor ANOVA was then performed, and it was found that there was a significant difference between sites low impact and high impact, medium impact and high impact, but no significance between sites low impact and medium impact (Table 3).

	Sites Low-Medium	Sites Medium-High	Sites Low-High
Variance	909.34	1064.3	1041.8
t-value	0.745	2.496	3.201
P-val (alpha = 0.0167)	0.457	0.0136	0.00165
Significant	no	yes	yes

Table 3. Multifactor ANOVA test to show presence or absence of statistical significance.

ANOVA tests were also performed for protozoa and fungi numbers between the three sites. Neither protozoa nor fungi had P values less than 0.05, meaning no significant difference between the three sites.

In addition to ANOVA tests, linear regressions were also run, by assigning 1, 2, and 3 as values to the pollution categorical (low, mid, high) variables. By linear regression, bacteria counts had a t-stat of 3.328, and a p-value of 0.001 (compared to a significance level of 0.05) and therefore had a statistically significant difference between sites. This reinforces the ANOVA and significance comparison between sites findings. Protozoa had a t-stat of -1.333, and a p-value of 0.184, and therefore was not statistically different between sites. Fungi had a t-stat of -0.3999, and a p-value of 0.690, and therefore was not statistically different between sites. Although Protozoa and Fungi did not have statistically significant differences between sites, one can notice that their values did result in a negative slope in the linear regression (and a corresponding negative t-stat).

Abundance & Biomass

Both sediment wet weight and dry weight biomass results are included in these results for reasons of error mentioned in the discussion. Between the three sites of low, medium, and high anthropogenic impact, a total of 493979 bacteria, 410 fungi, and 109 protozoa were observed.

For the site of low anthropogenic impact, a total of 147534 bacteria, 145 fungi, and 41 protozoa were observed. This was an average of 1821.41 bacteria, 1.79 fungi, and 0.51 protozoa per field of view under the microscope (Figure 2). In terms of biomass, this was an average of 3.15×10^{12} bacteria, 3.10×10^9 fungi, and 8.76×10^8 protozoa found per gram of wet sediment, and an average of 6.20×10^{12} bacteria, 6.10×10^9 fungi, and 1.72×10^9 protozoa found per gram of dry sediment (Table 4, Table 5).

For the site of medium anthropogenic impact, a total of 156209 bacteria, 127 fungi, and 38 protozoa were observed. This was an average of 1928.51 bacteria, 1.57 fungi, and 0.47 protozoa per field of view under the microscope (Figure 2). In terms of biomass, an average of 3.34×10^{12} bacteria, 2.71×10^9 fungi, and 8.12×10^8 protozoa were found per gram of wet sediment, and an average of 7.41×10^{12} bacteria, 6.03×10^9 fungi, and 1.80×10^9 protozoa were found per gram of dry sediment (Table 4, Table 5).

For the site of high anthropogenic impact, a total of 190236 bacteria, 138 fungi, and 30 protozoa were observed. This was an average of 2348.60 bacteria, 1.70 fungi, and 0.37 protozoa per field of view under the microscope (Figure 2). In terms of biomass, this was an average of 4.06×10^{12} bacteria, 2.96×10^9 fungi, and 6.41×10^8 protozoa found per gram of wet sediment, and an average of 5.90×10^{12} bacteria, 4.28×10^9 fungi, and 9.31×10^8 protozoa found per gram of dry sediment (Table 4, Table 5).

Average Number of Bacteria, Protozoa, and Fungi Seen in One Field of View in Three Sites

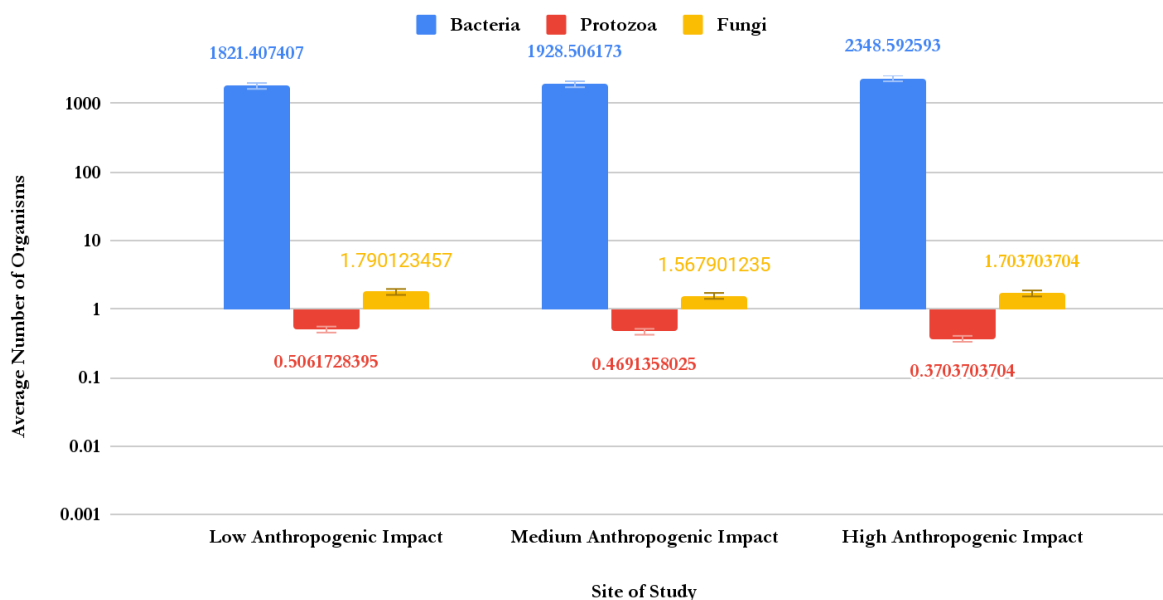


Figure 2. Average bacteria, protozoa, and fungi seen in one field of view under microscope at 100x in sites of low, medium, and high anthropogenic impact.

Wet weight

Site	Average Bacteria Observed per Gram of Moist Sediment	Average Fungi Observed per Gram of Sediment	Average Protozoa Observed per Gram of Sediment
Low Anthropogenic Impact	3.15×10^{12}	3.10×10^9	8.76×10^8
Medium Anthropogenic Impact	3.34×10^{12}	2.71×10^9	8.12×10^8
High Anthropogenic Impact	4.06×10^{12}	2.96×10^9	6.41×10^8

Table 4. Average biomass of bacteria, fungi, and protozoa per gram of wet sediment across sites of low, medium, and high anthropogenic impact.

Average Wet Weight Biomass of Bacteria, Fungi, and Protozoa Across Three Sites

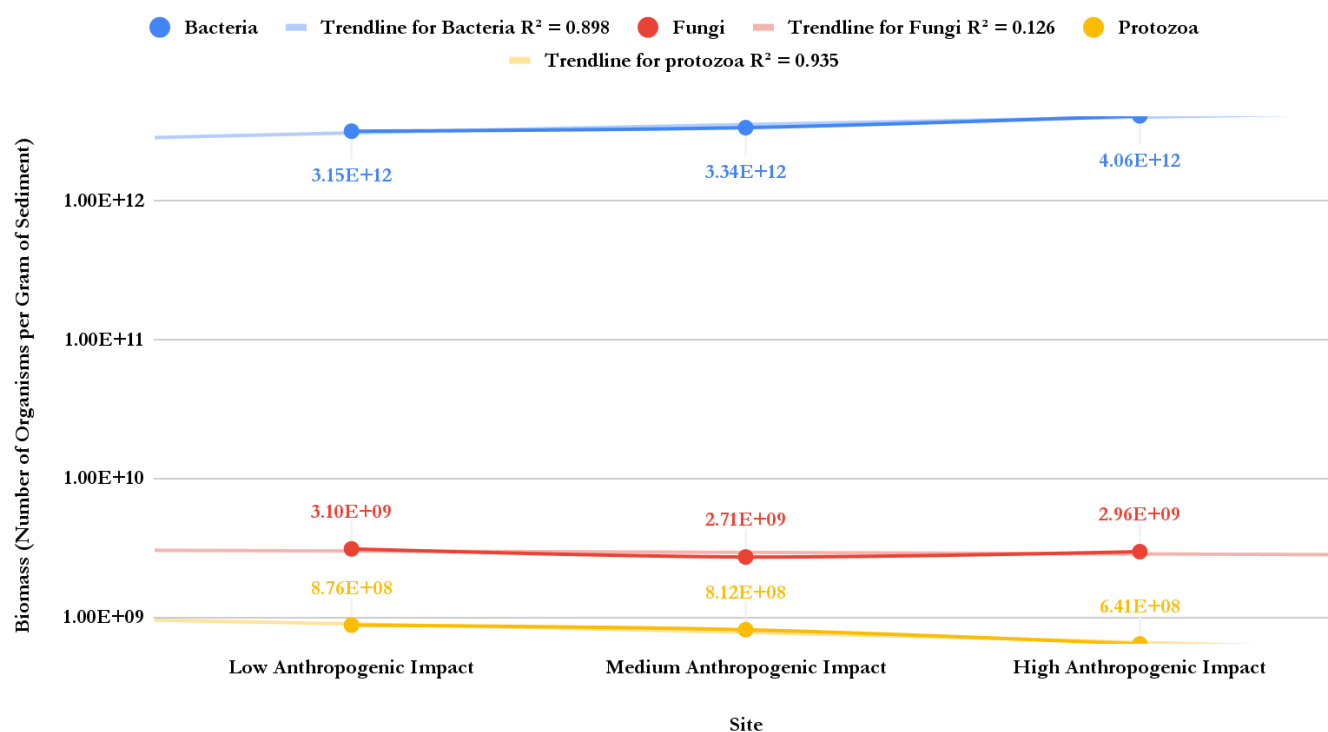


Figure 3. Average biomass of bacteria, fungi, and protozoa per gram of wet sediment across sites of low, medium, and high anthropogenic impact.

Dry weight

Site	Average Bacteria Observed per Gram of Sediment	Average Fungi Observed per Gram of Sediment	Average Protozoa Observed per Gram of Sediment
Low Anthropogenic Impact	6.20×10^{12}	6.10×10^9	1.72×10^9
Medium Anthropogenic Impact	7.41×10^{12}	6.03×10^9	1.80×10^9
High Anthropogenic Impact	5.90×10^{12}	4.28×10^9	9.31×10^8

Table 5. Average biomass of bacteria, fungi, and protozoa per gram of dry sediment across sites of low, medium, and high anthropogenic impact.

Average Dry Weight Biomass of Bacteria, Fungi, and Protozoa Across Three Sites

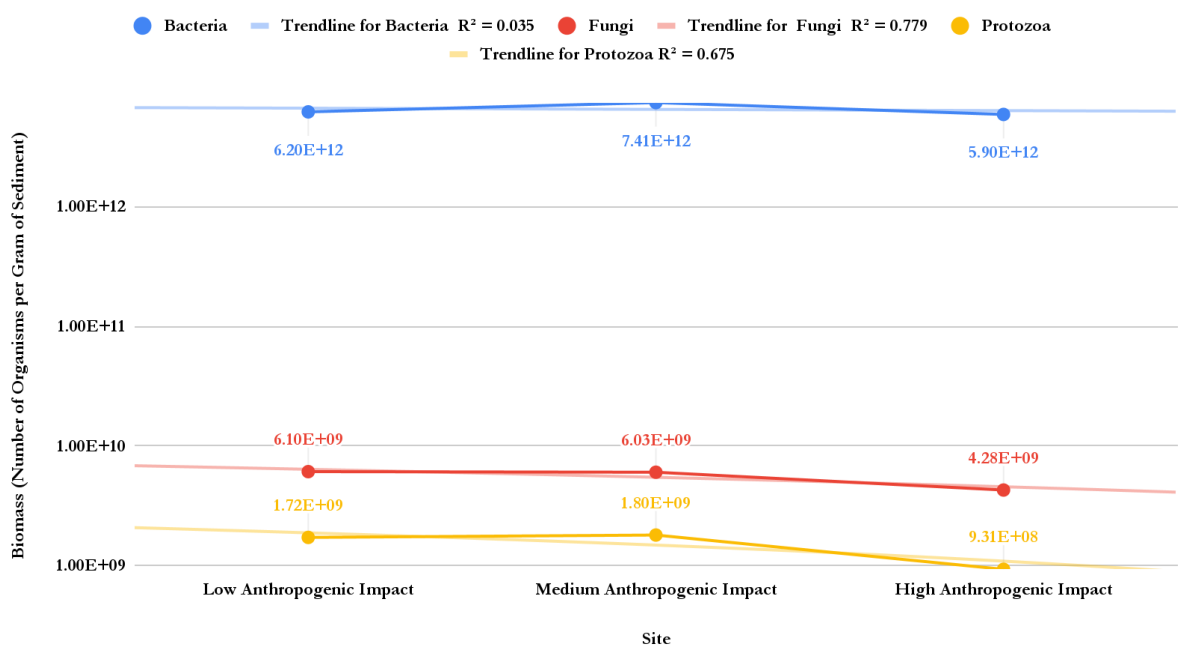


Figure 5. Average biomass of bacteria, fungi, and protozoa per gram of dry sediment across sites of low, medium, and high anthropogenic impact.

VIII. Discussion

Percent composition

The percent composition results are perhaps the most relevant to answering the question of the research objective. There is a small positive trend between bacteria percentage and pollution level. For each respective increase in anthropogenic influence level, the bacterial percentage went up by 0.02 percent points (Table 1, Figure 1). The trends for fungi and protozoa were less direct, but both demonstrated a negative correlation as anthropogenic impact levels increased (Figure 2). In essence, at higher pollution levels, bacteria were more dominant, even if by a small percentage, while fungi and protozoa percentages decreased. In terms of statistical significance, these results are difficult to make truly conclusive statements about, as the change between sites is so small. While the trend lines have statistically significant R^2 values, the scale of the change is extremely minute. Without further and more in depth studies spanning more sites, it is impossible to conclude if this relationship of percent composition with changing anthropogenic impact levels is at all truly significant.

The findings of this study of a shift in microbial composition between sites of varying pollution levels, while not necessarily statistically viable, do support past literature with these same conclusions (Chambers et al. 2016, Tso & Taghon 1997). There was a very minute directional change in microorganism percent makeup between the sites. Since this study was not performed in a controlled environment, this change could be attributed to many factors, but could possibly relate to the anthropogenic influence levels. As suggested in previous studies, this change of microbial makeup could be due to differences in the chemical and physical makeup of the sediment of interest (dos Santos et al. 2011, Hoshino et al. 2020), either naturally in place or created by human presence and contamination of the area. A previous study suggested changes in sediment microbial composition when waste water was introduced into the environment, which was a possible occurrence in the area of high anthropogenic impact (Saarenheimo et al. 2017).

Further studies would preferably be performed in a controlled environment in an effort to isolate and identify precisely which chemical and physical anthropogenic factors impact the microbial composition in mangrove sediment. This data could then be used in a similar manner as abundance data for assessing or monitoring sediment pollution levels over time. With a relationship of increasing bacterial dominance as anthropogenic impact increases, sediment human contamination changes over time or between sites could be calculated using this identified relationship.

While the numbers from this study do demonstrate a correlation in line with previously published literature, it is difficult to derive clear conclusions from this data about sediment microorganism composition. Despite the numerous samples collected throughout the three areas, many more than three areas would be needed to draw true conclusions about a consistent relationship between microorganism composition and pollution levels. The analysis would need to represent a clearer change in microorganism makeup between sites to provide any conclusive data. When reviewing this percent composition data, it is additionally important to keep in mind that only bacterial numbers had a statistical significance between sights, likely due to higher total bacterial counts. The percent composition data, while interesting for the research objective, can only be applied in a limited scope, and must be considered in terms of significance or lack thereof.

Diversity & Evenness

The diversity and evenness results align with percent composition data. As the bacterial dominance was higher as anthropogenic impact increased, it follows that the diversity and evenness in these sites would be lower. While many studies have not been performed on broader group diversity, past research has used bacterial diversity levels as indicators of marine sediment health (Ford et al.). In past studies, bacterial diversity was observed to be higher in sites of higher pollution (Sorci et al. 1999). It is not equivalent to apply this past research directly to the data from this project, but the lower diversity seen in sites of higher anthropogenic impact goes against the typical school of thought for assessing sediment health using diversity.

Diversity and evenness data would be of greater use when using more specific categories such as species. While this study did suggest the existence of differing diversity and evenness levels between the sites, it would be more specific and informative to perform diversity studies for each group (bacteria, protozoa, and fungi). With studies finding higher bacterial species richness in sites without pollution compared to those of high anthropogenic influence, future studies could compare the species richness of all three groups of bacteria, protozoa, and fungi (Ghizelini et al. 2019).

Abundance & Biomass

Based on the ANOVA test and the linear regression, the abundance results suggest only a significant difference in bacteria counts between sites. This may be understood simply through the amount of bacteria counted in comparison with the total other amounts of organisms seen. The much higher total count of bacteria provides a more statistically significant data set compared to protozoa and fungi. This result was somewhat expected, as bacteria are typically found in very high numbers in sediment, ranging from 2–1000 times more abundant in the sediment than surrounding water (Luo et al. 2019). Because of this numerical imbalance of bacteria in comparison to other organisms, it is hard to make any inferences about the meaning of the data between groups of organisms. While the objective for measuring abundance and biomass was to see if there was a relative change between the three groups of organisms between the 3 sites of pollution, a study of a much larger scale would be needed to make any conclusions about marine sediment fungi and protozoan abundance and biomass.

There were both average and total higher numbers of bacteria at higher levels of pollution. These findings go against some past literature, where bacterial and protozoa counts have both been found to be higher in non-polluted areas (Tso & Taghon 1997). The converse finding in this study could be explained by a variety of factors that were not controlled in this research, such as changes in sediment moisture between sites because of rain on various sample days, or unnoticed host forest composition. However, while not statistically significant, the protozoa abundance data supports this literature, as both total and average protozoa counts were higher in sites of lower anthropogenic impact. The fungi counts had no direct correlation with perceived pollution levels. Past studies have been in line with this finding, where no relationship between fungi abundance and other microbial abundance or sediment composition between individual sites was noted (Levine & Ghiorse 1990).

Further research into microorganism abundance in sites of differing anthropogenic impact is needed. With more concrete and numerous data, the correlation between bacterial abundance in mangrove sediment and anthropogenic impact could be used to assess the levels of human pollution in a specific site of interest. The bacterial counts could give evidence to changes in sediment pollution levels over time.

While biomass data is typically represented using dry weight of sediment, both were included in these results due to errors in finding dry weight. Because of the temporal, spatial, and funding limitations of this research, there was no method to uniformly dry the sediment cores. The sediments were set out to dry in aluminum trays for 2-3 days, but the weather differed greatly during the various drying times. When examining the calculations for dry biomass, each site lost varying amounts of water weight, despite the fact that the sediment samples were visually very similar. This is most likely an error due to the atmosphere the samples were dried in. For example, the samples from the area of medium impact lost an average of 55% of their weight when dried, but the samples from the high impact area lost an average of 31% of their weight when dried. This could be explained by the weather the high impact samples were dried in, which was very damp and rainy. The sample could not achieve a higher percent dried, despite water remaining, because of the humidity and lack of sun. This error ultimately would provide an inaccurate and inconsistent reflection of the true amount of dry sediment seen in each field of view in the microscope.

For these reasons, although lacking the accuracy that dry biomass would provide, the wet biomass data is likely more representative of the true biomass between the sites. While the wet weight biomass may not be exact, it is more consistent, and therefore more useful for comparing and understanding the results. The wet biomass numbers are also likely an underestimation of the true biomass of the microorganisms in the sediment, as this biomass would be including water in the sediment.

When using these parameters to consider the biomass results, some interesting data emerges. There was a trend of increasing bacterial biomass from going from low pollution to high pollution. There was also a trend of decreasing protozoa biomass going from low pollution to high pollution. This aligns with the composition percent results, and supports previous literature suggesting changes in both abundance and composition of microorganism communities between sites of differing anthropogenic impact (Tso & Taghon 1997).

IX. Conclusion

This study supports past literature in demonstrating differing microbial compositions between varying levels of anthropogenic impact. All of these conclusions apply only to these specific sites of study, as this experiment was not performed in a controlled environment, and was a very limited sample size. While not statistically significant, percent composition results provided the conclusion that bacteria increased in dominance over fungi and protozoa as perceived anthropogenic influences increased. This data was most relevant to answering the research question of a difference in microorganism makeup between sites. It was revealed that for these three sites, there was a small, but consistent change in microorganism composition. When considering abundance of bacteria, fungi, and protozoa, only bacterial counts were revealed to have a statistically significant (in terms of this study) difference between sites. It can be concluded that a higher abundance of bacteria was present at higher levels of anthropogenic impact. Biomass results also can be used to conclude that for these sites, bacteria and protozoa biomasses changed at a somewhat significant rate, while fungi biomass did not. The diversity and evenness also changed between the three sites, but also by very small factors. In all, there were seen to be multiple differences in sediment microorganism composition between the three sites of perceived low, medium, and high anthropogenic impact.

All of these conclusions, although relevant to these particular sites, do not hold statistical significance, as the study was not performed in a controlled environment. While the research

question was answered in terms of this site, these conclusions cannot be applied on a broader scale without further, controlled, and repeated studies. In future studies, anthropogenic factors could be isolated in a controlled setting to determine the effect on microbial communities. These studies could use this data to quantify and monitor human influence in various marine sediments of interest.

X. Works cited

- Allard, S. M., Costa, M. T., Bulseco, A. N., Helfer, V., Wilkins, L. G., Hassenrück, C., Zengler, K., Zimmer, M., Erazo, N., Mazza Rodrigues, J. L., Duke, N., Melo, V. M., Vanwonderghem, I., Junca, H., Makonde, H. M., Jiménez, D. J., Tavares, T. C., Fusi, M., Daffonchio, D., ... Bowman, J. (2020). Introducing the mangrove microbiome initiative: Identifying microbial research priorities and approaches to better understand, protect, and rehabilitate mangrove ecosystems. *MSystems*, 5(5). <https://doi.org/10.1128/msystems.00658-20>
- Alongi, D. M. (2012). Carbon sequestration in mangrove forests. *Carbon Management*, 3(3), 313–322. <https://doi.org/10.4155/cmt.12.20>
- Alongi, D. M. (2002). Present state and future of the world's mangrove forests. *Environmental Conservation*, 29(3), 331–349. <https://doi.org/10.1017/s0376892902000231>
- Al-Osta IM, Diab MS, Al-Shreef SAS (2021). ImageJ for Counting of Labeled Bacteria from Smartphone-Microscope Images. *J Mol Pharm Org Process Res* 9: 217. DOI: 10.4172/2329-9053.1000217
- Amend, A., Burgaud, G., Cunliffe, M., Edgcomb, V. P., Ettinger, C. L., Gutiérrez, M. H., Heitman, J., Hom, E. F., Ianiri, G., Jones, A. C., Kagami, M., Picard, K. T., Quandt, C. A., Raghukumar, S., Riquelme, M., Stajich, J., Vargas-Muñiz, J., Walker, A. K., Yarden, O., & Gladfelter, A. S. (2019). Fungi in the marine environment: Open questions and unsolved problems. *MBio*, 10(2). <https://doi.org/10.1128/mbio.01189-18>
- Chambers, L.G., Guevara, R., Boyer, J.N. et al. Effects of Salinity and Inundation on Microbial Community Structure and Function in a Mangrove Peat Soil. *Wetlands* 36, 361–371 (2016). <https://doi.org/10.1007/s13157-016-0745-8>
- Chen, M.-M., Nie, F.-H., Qamar, A., Zhu, D.-hua, Hu, Y., Zhang, M., Song, Q.-L., Lin, H.-Y., Chen, Z.-B., Liu, S.-Q., & Chen, J.-J. (2022). Effects of microplastics on microbial community in Zhanjiang mangrove sediments. *Bulletin of Environmental Contamination and Toxicology*, 108(5), 867–877. <https://doi.org/10.1007/s00128-021-03429-8>
- Chen, Q., Li, J., Zhao, Q., Jian, S., & Ren, H. (2018). Changes in the benthic protozoan community during succession of a mangrove ecosystem in Zhanjiang, China. *Ecosphere*, 9(4). <https://doi.org/10.1002/ecs2.2190>
- Chen, Q.-H., Xu, R.-L., Tam, N. F. Y., Cheung, S. G., & Shin, P. K. S. (2008). Use of ciliates (protozoa: Ciliophora) as bioindicator to assess sediment quality of two constructed mangrove sewage treatment belts in southern China. *Marine Pollution Bulletin*, 57(6-12), 689–694. <https://doi.org/10.1016/j.marpolbul.2008.03.015>
- Cheung, M.K., Wong, C.K., Chu, K.H. et al. Community Structure, Dynamics and Interactions of Bacteria, Archaea and Fungi in Subtropical Coastal Wetland Sediments. *Sci Rep* 8, 14397 (2018). <https://doi.org/10.1038/s41598-018-32529-5>

- Chmura, G. L., Anisfeld, S. C., Cahoon, D. R., & Lynch, J. C. (2003). Global carbon sequestration in Tidal, Saline Wetland soils. *Global Biogeochemical Cycles*, 17(4). <https://doi.org/10.1029/2002gb001917>
- Collin, R. (2005). Ecological Monitoring and Biodiversity Surveys at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station. *Caribbean Journal of Science*, 41(3).
- Dimitriu, P.A., Grayston, S.J. Relationship Between Soil Properties and Patterns of Bacterial β -diversity Across Reclaimed and Natural Boreal Forest Soils. *Microb Ecol* 59, 563–573 (2010). <https://doi.org/10.1007/s00248-009-9590-0>
- dos Santos, H. F., Cury, J. C., do Carmo, F. L., dos Santos, A. L., Tiedje, J., van Elsas, J. D., Rosado, A. S., & Peixoto, R. S. (2011). Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: Bacterial proxies for oil pollution. *PLoS ONE*, 6(3). <https://doi.org/10.1371/journal.pone.0016943>
- Feller, I. C., Lovelock, C. E., Berger, U., McKee, K. L., Joye, S. B., & Ball, M. C. (2010). Biocomplexity in mangrove ecosystems. *Annual Review of Marine Science*, 2(1), 395–417. <https://doi.org/10.1146/annurev.marine.010908.163809>
- Finlay, B. J. (1998). The global diversity of protozoa and other small species. *International Journal for Parasitology*, 28(1), 29–48. [https://doi.org/10.1016/s0020-7519\(97\)00167-7](https://doi.org/10.1016/s0020-7519(97)00167-7)
- Ford, T., Jay, J., Patel, A., Kile, M., Prommasith, P., Galloway, T., Sanger, R., Smith, K., & Depledge, M. (2005). Use of ecotoxicological tools to evaluate the health of New Bedford Harbor Sediments: A microbial biomarker approach. *Environmental Health Perspectives*, 113(2), 186–191. <https://doi.org/10.1289/ehp.6934>
- Ghizelini, A. M., Martins, K. G., Giebelmann, U. C., Santoro, E., Pasqualetto, L., Mendonça-Hagler, L. C. S., Rosado, A. S., & Macrae, A. (2019). Fungal communities in oil contaminated mangrove sediments – who is in the mud? *Marine Pollution Bulletin*, 139, 181–188. <https://doi.org/10.1016/j.marpolbul.2018.12.040>
- Ghizelini, A. M., Mendonça-Hagler, L. C., & Macrae, A. (2012). Microbial diversity in Brazilian mangrove sediments: A mini review. *Brazilian Journal of Microbiology*, 43(4), 1242–1254. <https://doi.org/10.1590/s1517-83822012000400002>
- Goldberg, L., Lagomasino, D., Thomas, N., & Fatoyinbo, T. (2020). Global declines in human-driven mangrove loss. *Global Change Biology*, 26(10), 5844–5855. <https://doi.org/10.1111/gcb.15275>
- Granek, E., & Ruttenger, B. I. (2008). Changes in biotic and abiotic processes following mangrove clearing. *Estuarine, Coastal and Shelf Science*, 80(4), 555–562. <https://doi.org/10.1016/j.ecss.2008.09.012>

- Haldar, S., & Nazareth, S. W. (2018). Taxonomic diversity of bacteria from mangrove sediments of goa: Metagenomic and functional analysis. *3 Biotech*, *8*(10). <https://doi.org/10.1007/s13205-018-1441-6>
- Harborne, A. R., Mumby, P. J., Micheli, F., Perry, C. T., Dahlgren, C. P., Holmes, K. E., & Brumbaugh, D. R. (2006). The functional value of Caribbean coral reef, seagrass and mangrove habitats to ecosystem processes. *Advances in Marine Biology*, *57*–189. [https://doi.org/10.1016/s0065-2881\(05\)50002-6](https://doi.org/10.1016/s0065-2881(05)50002-6)
- Hoshino, T., Doi, H., Uramoto, G.-I., Wörmer, L., Adhikari, R. R., Xiao, N., Morono, Y., D'Hondt, S., Hinrichs, K.-U., & Inagaki, F. (2020). Global diversity of microbial communities in marine sediment. *Proceedings of the National Academy of Sciences*, *117*(44), 27587–27597. <https://doi.org/10.1073/pnas.1919139117>
- Jennerjahn, T. C. (2021). Relevance and magnitude of 'blue carbon' storage in mangrove sediments: Carbon accumulation rates vs. stocks, sources vs. sinks. *Estuarine, Coastal and Shelf Science*, *248*, 107156. <https://doi.org/10.1016/j.ecss.2020.107156>
- Kayes, Rebecca, "Coral Reef Tourism and Conservation in Bocas del Toro: An Analysis of Ecotourism and its Tour Guide-Based Components" (2005). Independent Study Project (ISP) Collection. 433. https://digitalcollections.sit.edu/isp_collection/433
- Kim, J., Lee, J., Yang, Y., Yun, J., Ding, W., Yuan, J., Khim, J. S., Kwon, B.-O., & Kang, H. (2021). Microbial decomposition of soil organic matter determined by edaphic characteristics of mangrove forests in East Asia. *Science of The Total Environment*, *763*, 142972. <https://doi.org/10.1016/j.scitotenv.2020.142972>
- Lee, N. L., Huang, D., Quek, Z. B., Lee, J. N., & Wainwright, B. J. (2019). Mangrove-associated fungal communities are differentiated by geographic location and host structure. *Frontiers in Microbiology*, *10*. <https://doi.org/10.3389/fmicb.2019.02456>
- Levine, S. N., & Ghiorse, W. C. (1990). Environmental factors affecting distribution and abundance of bacteria, fungi and protozoa in subsurface sediments of the Upper Atlantic Coastal Plain, USA. *University of North Texas Libraries*.
- Lovelock, C. E., Feller, I. C., McKee, K. L., & Thompson, R. C. (2005). Variation in mangrove forest structure and sediment characteristics in Bocas del Toro, Panama. *Caribbean Journal of Science*.
- Lowy, F. (2009). *Bacterial classification, Structure and Function - Columbia University*. Retrieved from <http://www.columbia.edu/itc/hs/medical/pathophys/id/2009/introNotes.pdf>
- Luo, X., Xiang, X., Huang, G., Song, X., Wang, P., & Fu, K. (2019). Bacterial abundance and physicochemical characteristics of water and sediment associated with hydroelectric dam on the Lancang River China. *International Journal of Environmental Research and Public Health*, *16*(11), 2031. <https://doi.org/10.3390/ijerph16112031>

- Macreadie, P. I., Anton, A., Raven, J. A., Beaumont, N., Connolly, R. M., Friess, D. A., Kelleway, J. J., Kennedy, H., Kuwae, T., Lavery, P. S., Lovelock, C. E., Smale, D. A., Apostolaki, E. T., Atwood, T. B., Baldock, J., Bianchi, T. S., Chmura, G. L., Eyre, B. D., Fourqurean, J. W., ... Duarte, C. M. (2019). The Future of Blue Carbon Science. *Nature Communications*, 10(1). <https://doi.org/10.1038/s41467-019-11693-w>
- Madrid, R. E., & Felice, C. J. (2005). Microbial Biomass estimation. *Critical Reviews in Biotechnology*, 25(3), 97–112. <https://doi.org/10.1080/07388550500248563>
- Nealson, K. H. (1997). Sediment bacteria: Who's there, what are they doing, and what's new? *Annual Review of Earth and Planetary Sciences*, 25(1), 403–434. <https://doi.org/10.1146/annurev.earth.25.1.403>
- Padma Dorothy, K., Satyanarayana, B., Kalavati, C., Raman, A. V., & Dehairs, F. (2003). Protozoa associated with leaf litter degradation in Coringa mangrove forest, Kakinada Bay, east coast of India. *Indian Journal of Marine Sciences*, 32(1).
- Pitt, T. L., & Barer, M. R. (2012). Classification, identification and typing of micro-organisms. *Medical Microbiology*, 24–38. <https://doi.org/10.1016/b978-0-7020-4089-4.00018-4>
- Pratt, J., & Cairns, J. (1985). Functional groups in the Protozoa: Roles in differing ecosystems^{1,2}. *The Journal of Protozoology*, 32(3), 415–423. <https://doi.org/10.1111/j.1550-7408.1985.tb04037.x>
- Preparing a wet mount - Centers for Disease Control and Prevention*. (n.d.). Retrieved from https://www.cdc.gov/labtraining/docs/job_aids/routine_microscopy_procedures/Preparing-a-Wet-Mount_508.pdf
- Protocol: Seagrass Core Biomass (2020) Tennenbaum Marine Observatories Network, MarineGEO, Smithsonian Institution. Retrieved from https://marinegeo.github.io/assets/modules/seagrass-biomass/marinegeo_protocol_seagrass_biomass.pdf
- Romañach, S. S., DeAngelis, D. L., Koh, H. L., Li, Y., Teh, S. Y., Raja Barizan, R. S., & Zhai, L. (2018). Conservation and restoration of mangroves: Global Status, Perspectives, and Prognosis. *Ocean & Coastal Management*, 154, 72–82. <https://doi.org/10.1016/j.ocecoaman.2018.01.009>
- Saarenheimo, J., Aalto, S. L., Rissanen, A. J., & Tirola, M. (2017). Microbial community response on wastewater discharge in Boreal Lake Sediments. *Frontiers in Microbiology*, 8. <https://doi.org/10.3389/fmicb.2017.00750>
- Sasmito, S. D., Kuzyakov, Y., Lubis, A. A., Murdiyarso, D., Hutley, L. B., Bachri, S., Friess, D. A., Martius, C., & Borchard, N. (2020). Organic Carbon Burial and sources in soils of coastal mudflat and mangrove ecosystems. *CATENA*, 187, 104414. <https://doi.org/10.1016/j.catena.2019.104414>

- Schmid, R., & Tomlinson, P. B. (1987). The botany of mangroves. *Taxon*, 36(1), 304.
<https://doi.org/10.2307/1221417>
- Shiau, Y.-J., & Chiu, C.-Y. (2020). Biogeochemical processes of C and N in the soil of mangrove forest ecosystems. *Forests*, 11(5), 492. <https://doi.org/10.3390/f11050492>
- Sims, A., Zhang, Y., Gajaraj, S., Brown, P. B., & Hu, Z. (2013). Toward the development of Microbial Indicators for wetland assessment. *Water Research*, 47(5), 1711–1725. <https://doi.org/10.1016/j.watres.2013.01.023>
- Smith, A. C., & Hussey, M. A. (2005, September 30). Gram stain protocols - American Society for Microbiology. Retrieved from
<https://asm.org/getattachment/5c95a063-326b-4b2f-98ce-001de9a5ece3/gram-stain-protocol-2886.pdf>
- Sorci, J. J., Paulauskis, J. D., & Ford, T. E. (1999). 16S rRNA restriction fragment length polymorphism analysis of bacterial diversity as a biomarker of ecological health in polluted sediments from New Bedford Harbor, Massachusetts, USA. *Marine Pollution Bulletin*, 38(8), 663–675. [https://doi.org/10.1016/s0025-326x\(98\)90199-0](https://doi.org/10.1016/s0025-326x(98)90199-0)
- Treves, D., Xia, B., Zhou, J. et al. A Two-Species Test of the Hypothesis That Spatial Isolation Influences Microbial Diversity in Soil. *Microb Ecol* 45, 20–28 (2003).
<https://doi.org/10.1007/s00248-002-1044-x>
- Troxler, T.G., Ikenaga, M., Scinto, L. et al. Patterns of Soil Bacteria and Canopy Community Structure Related to Tropical Peatland Development. *Wetlands* 32, 769–782 (2012).
<https://doi.org/10.1007/s13157-012-0310-z>
- Tso, S. F., & Taghon, G. L. (1997). Enumeration of protozoa and bacteria in muddy sediment. *Microbial Ecology*, 33(2), 144–148. <https://doi.org/10.1007/s002489900016>
- Valiela, I., Bowen, J., & York, J. (2001). Mangrove forests: One of the world's threatened Major Tropical Environments. *BioScience*, 51(10), 807.
[https://doi.org/10.1641/0006-3568\(2001\)051\[0807:mfootw\]2.0.co;2](https://doi.org/10.1641/0006-3568(2001)051[0807:mfootw]2.0.co;2)
- van Beelen, P., & Doelman, P. (1997). Significance and application of microbial toxicity tests in assessing ecotoxicological risks of contaminants in soil and sediment. *Chemosphere*, 34(3), 455–499. [https://doi.org/10.1016/s0045-6535\(96\)00388-8](https://doi.org/10.1016/s0045-6535(96)00388-8)
- Wang, L., Huang, X., & Zheng, T. L. (2016). Responses of bacterial and archaeal communities to nitrate stimulation after oil pollution in mangrove sediment revealed by Illumina sequencing. *Marine pollution bulletin*, 109(1), 281–289.
<https://doi.org/10.1016/j.marpolbul.2016.05.068>
- Widenfalk, A., Bertilsson, S., Sundh, I., & Goedkoop, W. (2008). Effects of pesticides on community composition and activity of sediment microbes – responses at various levels of microbial community organization. *Environmental Pollution*, 152(3), 576–584.
<https://doi.org/10.1016/j.envpol.2007.07.003>

Zhuang, W., Yu, X., Hu, R. et al. Diversity, function and assembly of mangrove root-associated microbial communities at a continuous fine-scale. *npj Biofilms Microbiomes* 6, 52 (2020). <https://doi.org/10.1038/s41522-020-00164-6>

XI. Appendix

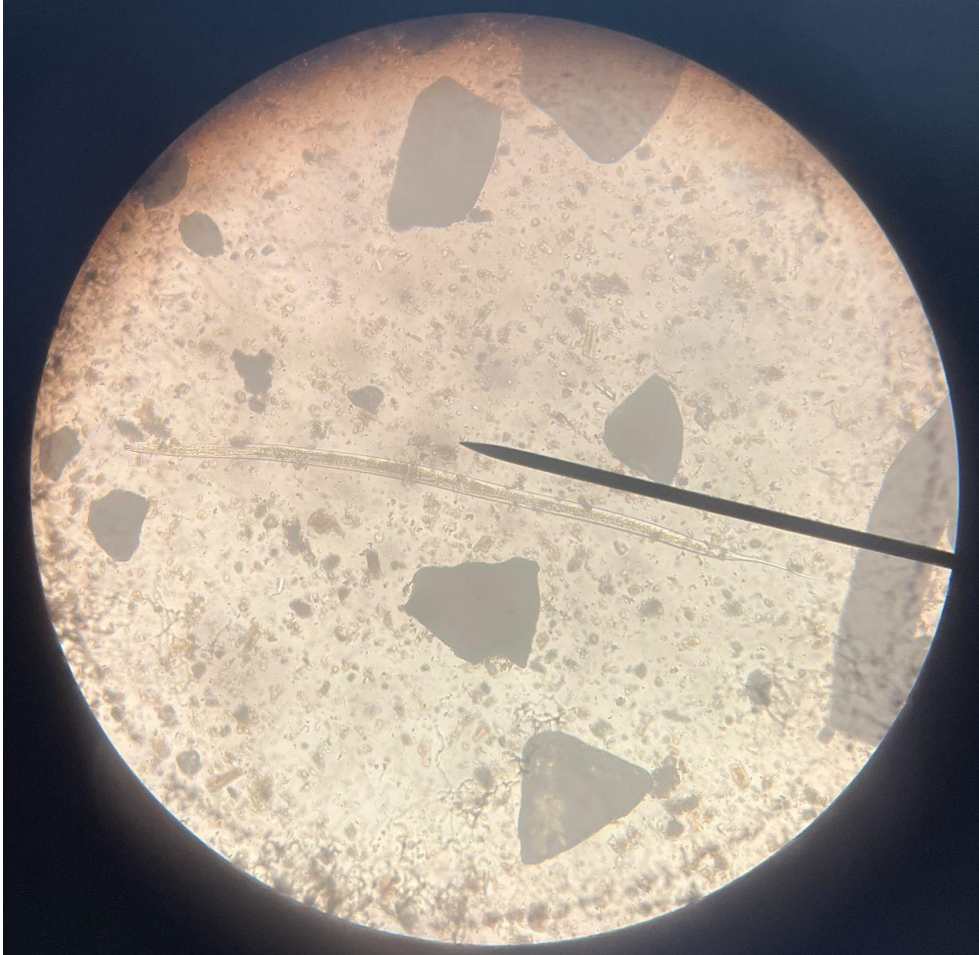


Figure 6. Example of a protozoa (nematode) observed at 100x. Fungi and bacteria can also be observed.

Volume of Sample in Field of View Calculations

To calculate the “thickness” of the sample in the field of view:

Volume of drop: 0.05 mL

Size of coverslip: 22 mm x 22 mm

→ From observation, and for the purpose of this research, assuming drop fills bounds of coverslip

$$V = wLh$$

$$.05 = 22 * 22 * h$$

$$h = .0001 \text{ mm}$$

“Thickness” = approx 1 micrometer

→ Makes sense: one layer of bacteria, bacteria approx 1 micrometer in size

To calculate volume in field of view

At 100x magnification, the field of view has a diameter of .2 mm

$$V = \frac{4}{3} \pi * L * w * h$$

$$V = \frac{4}{3} \pi * .2 * .2 * .0001$$

$$V = 1.675 \times 10^{-5} \text{ mm}^3$$

$$V = 1.675 \times 10^{-8} \text{ mL}$$

To calculate amount of sediment in this

Samples were diluted 5 g sample → 145 ml water

$$5/145 = x / 1.675 \times 10^{-8}$$

$$x = 5.78 \times 10^{-10} \text{ g of sediment per field of view}$$